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Evaluating Ligand-Receptor Networks of TGF-β with Membrane Computing

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Abstract: Ligand-Receptor Networks of TGF-β plays essential role in transmitting a wide range of extracellular signals that affect many cellular processes such as cell growth. However, the modeling of these networks with conventional approach such as ordinary differential equations has not taken into account, the spatial structure and stochastic behavior of processes involve in these networks. Membrane computing as the alternatives approach provides spatial structure for molecular computation in which processes are evaluated in a non-deterministic and maximally parallel way. This study is carried out to evaluate the membrane computing model of Ligand-Receptor Networks of TGF-β with model checking approach. The results show that membrane computing model has sustained the behaviors and properties of Ligand-Receptor Networks of TGF-β. This reinforce that membrane computing is capable in analyzing processes and behaviors in hierarchical structure of cell such as Ligand-Receptor Networks of TGF-β better than the deterministic approach of conventional mathematical models.

Key words: System biology, cellular processes, membrane computing, model checker

INTRODUCTION

Transforming Growth Factor Beta (TGF-β) is a type of protein that produced by cells (Shi and Massague, 2003). Cells also generate receptors for the functions of TGF-β. In tissue homeostasis and morphogenesis, TGF-β signal transduction pathway plays a crucial role to control the excess of cellular processes such as cell growth (Massagu, 1998). Complex signal transduction mechanism incorporates signals from various ligands of the TGF-β superfamily at the molecular level (Shi and Massague, 2003). This mechanism also integrates the component of the two main categories of receptors which are known as type-1 and type-2 receptors (Massagu, 1998). With the both receptors, each ligand stimulates the formation of a receptor complex which then transmits through the membranes. The capacity of ligands to bind several type-1 and type-2 receptors leads to a complex ligand-receptor interaction network.

TGF-β has particular interest in cancer research (Steiner and Barrack, 1992) in which, it suppresses cellular growth and its inactivation contributes to tumourigenesis in epithelial cells. The versatility of the network in eliciting various types of behaviour during tumour progression is characterized by the ability of TGF-β to transform its function from suppressor to promoter of growth in epithelial cells (Steiner and Barrack, 1992). TGF-β could also suppress the growth of cells around the tumour to shut down the local immune system to encourage angiogenesis (Steiner and Barrack, 1992). These paracrine signalling would affect the growth of the tumour *in vivo* (Steiner and Barrack, 1992).

The structure and network of Ligand-Receptor Networks of TGF-β have to be taken into account when modeling the system. The computation model of Ordinary Differential Equations (ODE) by Vilar *et al*. (2006) has been used to analyze the signal processing in Ligand-Receptor Networks of TGF-β by measuring the ligand and receptors concentration in the system. With this approach, the general behaviors of the system were investigated with deterministic evaluation of the processes but disregarded the system structure and stochastic behaviors involved in processes on the molecular level (De Jong, 2002).

Membrane computing (Paun, 2000) which inspired by the hierarchical structure of living cells could address such limitations (Muniyandi and Zin, 2009). This is mainly because the ability of membrane computing to model molecular computing by providing a spatial structure for molecular computation.

In the structure and the functioning of cell, membranes play a crucial role in which objects pass through, in a coordinated way within and across the membranes. The cell is encapsulated from its environment by plasma membrane and it is compartmentalized by internal membranes. The membrane computing model

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formalizes this fundamental feature of the living cell, namely, membrane structure (Paun, 2000).

Membrane computing is applied in parallel, distributed and nondeterministic way which means all the objects that can evolve must evolve (Paun, 2000). The fundamental conceptions that are employed in this computing model are a membrane structure in which objects evolve in accordance with specified evolution rules.

This study describes the evaluation of the membrane computing model of Ligand-Receptor Networks of TGF-β by using model checking approach. The properties of Ligand-Receptor Networks of TGF-β are used in the evaluation processes.

MATERIALS AND METHODS

Membrane computing model: The membrane computing model of Ligand-Receptor Networks of TGF-β is given in Muniyandi and Zin (2011). The model is summarized as follow:

The membrane system of ligand receptor network of TGF-β (LRN) is represented as:

$$LRN = (V, \mu, \omega_P, \omega E, R_P, R_E)$$

The system contains two compartments that are Plasma membrane (P) and Endosome (E). Its membrane structure is represented as:

$$\mu = [[]_E]_P$$

There are four objects which are receptors and complex of ligand and receptors. l is the ligand, RI and RII are receptors and lRIRII is ligand-receptors complex. The objects are listed as follow:

$$V = \{l, Rl, Rll, lRIRII\}$$

The multisets in P and E at time step t = 0 are:

$$\omega_P = \{l, Rl, Rll\} \text{ and } \omega_E = \{lRlRll\}$$

The evolution rule has the form:

$$R_n : \omega_1[\omega_2]_n \xrightarrow{\alpha} \omega_3[\omega_4]_n$$

where, ω_1, ω_2, ω_3, ω_4 are multisets and n is the label of compartment. α is a real number representing the kinetic constant. The rules in Ligand-Receptor Networks of TGF-β are represented in membrane computing as follow:

- Formation of ligand receptor complex:

$$R_p1:[l,RI,RII]_P \xrightarrow{k_a} [lRIRII,l]_P$$

- Constitutive degradation of ligand receptor complex:

$$R_p2:[lRIRII]_P \xrightarrow{k_{cd}} [\,]_P$$

- Independent complex degradation of ligand:

$$R_p3:[lRIRII]_P \xrightarrow{k_{id}} [\,]_P$$

- Internalization of ligand receptor complex:

$$R_p4:lRIRII[\,]_E \xrightarrow{k_i} [lRIRII]_E$$

- Synthesis of RI:

$$R_p5:[\,]_P \xrightarrow{P_{RI}} [RI]_P$$

- Constitutive degradation of RI:

$$R_p6:[RI]_P \xrightarrow{K_{cd}} [\,]_P$$

- Internalization of RI:

$$R_p7:RI[\,]_E \xrightarrow{K_i} [RI]_E$$

- Recycling of RI:

$$R_E1:[RI]_E \xrightarrow{K_r} RI[\,]_E$$

- Recycling of ligand Receptor complex:

$$R_E2:[lRIRII]_E \xrightarrow{K_r} lRIRII[\,]_E$$

$$R_P8:[lRIRII]_P \xrightarrow{K_r\alpha} [RI,RII]_P$$

- Synthesis of RII:

$$R_P9:[\,]_P \xrightarrow{P_{RII}} [RII]_P$$

- Constitutive degradation of RII:

$$R_P10:[RII]_P \xrightarrow{k_{cd}} [\,]_P$$

- Internalization of RII:

$$R_P11:RII[\,]_E \xrightarrow{k_i} RII[\,]_E$$

- Recycling of RII:

$$R_E3:[RII]_E \xrightarrow{k_r} RII[\,]_E$$

Properties of Ligand-Receptor Networks of TGF-β: The aim of membrane computing model is to investigate the signaling activity of the ligand receptor networks. The number of ligand receptor complexes activated in the internalized endosomes is proportional to the signaling activity of the ligand receptor networks. The other essential properties (Vilar *et al*., 2006) that required for this model to preserve the behavior of the system are:

- **Property (1):** With RI and RII receptors, ligands stimulate the formation of receptor complexes
- **Property (2):** Receptors and ligand-receptor complexes are constantly available in both plasma membrane and internalized endosomes
- **Property (3):** The internalization into endosomes and recycling into the plasma membrane of receptors and ligand-receptor complexes are applied continuously
- **Property (4):** The receptors and ligand-receptor complexes has a constitutive contribution through receptor degradation
- **Property (5):** Receptors that have been complexes with ligands are affected by ligand-induced contribution through receptor degradation

Model checker: The model checking approach is utilized to validate membrane computing model. In modeling and verification tasks, the concepts of model validity, verification, credibility and tractability are of great importance (Raczynski, 2006). Meanwhile, model checking is implemented on a model of a system by testing automatically to determine whether this model fulfill a given specification or properties. Model checking is one of the leading applications of logic to computer science and it has attained many advances, bridging the gap between theoretical computer science and, hardware and software engineering and it is getting into new fields such as system biology and hybrid systems (Grumberg and Veith, 2008). In this study Probabilistic and Symbolic Model Checker (PRISM) (Kwiatkowska *et al*., 2002) is utilized as a tool for stochastic model checking. The approach to analyze the reliability and performance of a stochastic system required the capabilities to verify the properties or performance of the system. In stochastic modeling, Continuous Time Markov Chain (CTMC) is a method that uses Continuous Stochastic Logic (CSL) which allows assigning properties over states as well as paths (Kuntz and Siegle, 2006). PRISM is based on CTMC and CSL for stochastic systems. Besides the stochastic elements, PRISM also supports other characteristics of membrane computing such as hierarchical compartments, parallel behavior, communication mechanism, multisets of objects and transition coverage. Other than those advantages of PRISM, it also has the automatic reasoning capabilities required for stochastic systems and with this characteristic it can be used to validate the properties of biological systems. For the model checking purposes, the specific properties or behaviors of the biological systems are identified and expressed as temporal logic formulas. PRISM utilized efficient symbolic algorithms which able to traverse the membrane computing model and check if the specification holds or not. PRISM is based on the reactive modules formalism (Alur and Henzinger, 1999) which is a simple, high level and state-based language. Membrane computing model is specified in PRISM to be model checked. The model checking approach for membrane computing model of biological system is described by Muniyandi *et al*. (2010).

Translation of membrane computing into PRISM: The method of conversion from membrane computing into PRISM is given by Romero-Campero *et al*. (2006). The membrane structure is represented by using modules and, the rules and objects within membrane are represented with commands and local variables as follow:

- The membrane i is represented with a module called compartment_i
- The object $o \in V$ is represented as local variable and it is denoted as o_i in compartment_i. The initial multiset ω_i determines the initial value of the variable. The value range of the variable is declared as a constant o_i_bound to represent the upper bound of the object o_i
- The rule R_i is described as command. The evolution rule can be divided into two based on its features. The first is communication rule when there is exchange of object between two membranes and the second is transformation rule when the object transforms to one to another within the membrane
- Communication rule needs two membranes to exchange the objects in a synchronized way. Assuming that compartment j is encapsulated in compartment i and the synchronized rule R_p (p is the index of the rule) is:

$$R_p : u_1,...,u_q[v_1,...,v_r]_j \xrightarrow{k} x_1,...,x_s[y_1,....,y_t]_j$$

The prism command in module compartment_i is:

```
[rule_p] u1_i > 0 & ... & uq_i > 0 &
    x1_i < x1_i_bound & ... & xs_i < xs_i_bound →
    k * u1_i * ... *uq_i :
    (u1_i' = u1_i - 1) & ... & (uq_i' = uq_i - 1) &
(x1_i' = x1_i + 1) & ... & (xs_i' = xs_i + 1);
```

The command in module compartment_j is:

```
[rule_p] v1_j > 0 & ... & vr_j > 0 &
y1_j < y1_j_bound & ... & yt_j < yt_j_bound →
v1_j * ... * vr_j :
(v1_j' = v1_j - 1) & ... & (vr_j' = vr_j - 1) &
(y1_j' = y1_j + 1) & ... & (yt_j' = yt_j + 1);
```

When the condition in guards is true, these two commands are applied simultaneously. The product of the individual rates is the rate of this transition:

$$(k * u1\_i * ... * uq\_i) * (v1\_j * ... * vr\_j)$$

In transformation rule no synchronization is needed. The rule R_p is:

$$R_p : [u_1, ..., u_q]_i \xrightarrow{k} [v_1, ..., v_r]_j$$

The PRISM command is:

```
[] u1_i > 0 &... & uq_i > 0 &
v1_i < v1_i_bound & ... & vr_i < vr_i_bound →
k * u1_i * ... * uq_i :
(u1_i' = u1_i - 1) & ... & (uq_i' = uq_i - 1) &
(v1_i' = v1_i + 1) & ... & (vr_i' = svr_i + 1)
```

Property specifications: The model in PRISM is validated with the properties of the system as explained by Kwiatkowska *et al*. (2007). PRISM property specification is implemented by using mainly three types of operators, the P operator, S operator and R operator. Probability of an event's occurrence is analyzed by using P operator. The steady-state behavior of a model is reasoned by using S operator. The R operator is used to analyze the rewards based properties. In this investigation, rewards based properties are used for property specification. In rewards based reasoning, the behavior of the model is analyzed by measuring the probability as well as by classifying a wide range of quantitative measures that involving modeling behavior. For instance, for the molecular reaction system, the following reward is specified to measure the percentage of Na molecules present in the system with N1 as the initial amount of Na.

```
rewards "percentage_Na"
true: 100*Na/N1;
endrewards
```

RESULTS

PRISM is used to evaluate the properties of Ligand-Receptor Networks of TGF-β by using the concept of rewards as follow.

Property (1): The process of ligands inducement with RI and RII receptors in the formation of receptor complexes IRIRII is measured with rewards CF. It evaluates the IRIRII formation at each time step in plasma membrane. Figure 1 demonstrates the evaluation of this property based on rewards CF. The figure shows that the formation of IRIRII in plasma membrane is more frequently activated at the initial stage of this reaction. At this stage internalization into endosomes is occurring consistently but slowly as shown in the simulation done by Muniyandi and Zin (2011). The simulation also showed that between the time steps of 2000 and 3000, internalization is rapidly activated revealed by the peak achieved by the IRIRII in endosome. Subsequently, the formation of IRIRII in plasma membrane is becoming steady with the combination of recycling and internalization. This shows that the consistent availability of ligands induces the formation of lRIRII according to the amount of RI and RII receptors present in plasma membrane at each time steps.

Property (2): Six rewards are used to measure the availability of RI, RII and IRIRII in both plasma membrane and endosome.

Figure 2 and 3 show the concentration of RI, RII and IRIRII in plasma membrane and endosomes. This demonstrates that at each time steps receptors and ligand receptor complexes present in both compartments in distinct amounts. IRIRII concentration in endosomes is used to evaluate signal processing which requires the interaction among the receptors and ligand receptor complexes in these two compartments. This network is materialized by internalization of RI and RII from plasma membrane to increase the concentration of these receptors in endosomes which in turn enhance the concentration of IRIRII until it lead to a peak. But the concentration of receptors and ligand receptor complexes are becoming steady with recycling and internalization processes after the peak. This shows that, receptors and ligand-receptor complexes are present in plasma membrane and endosomes to sustain the processes in the system.

Property (3, 4): Rewards IN determines the internalization of RI, RII and IRIRII into endosomes. Rewards RC measures the recycling back of RI, RII and IRIRII into the plasma membrane. The receptors and ligand-receptor complexes are consistently internalized into endosomes and recycled into plasma membrane as shown in Fig. 4 and 5. Before achieving the peak for IRIRII in endosomes, the internalization process is relatively slow. Almost not much recycling activity at the initial stage before gaining momentum after IRIRII achieves the peak in endosomes. Both internalization and recycling activities become constant to stabilize the system at last.

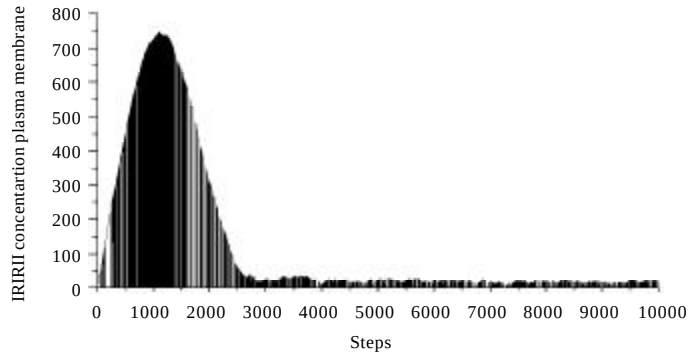

Fig. 1: IRIRII formation in plasma membrane

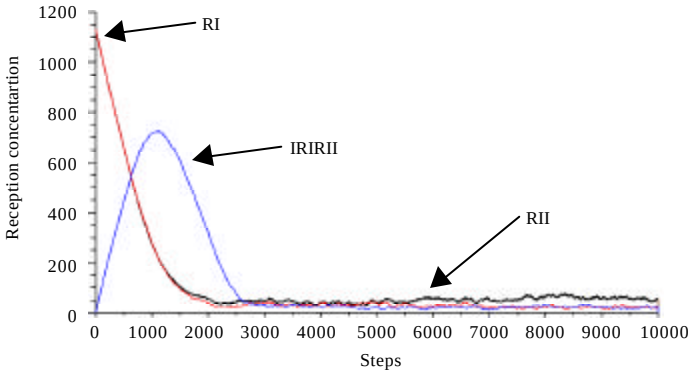

Fig. 2: RI, RII and IRIRII in plasma membrane

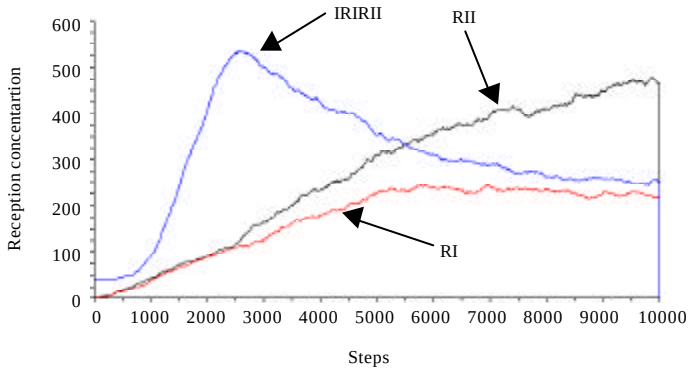

Fig. 3: RI, RII and IRIRII in endosomes

Property (5): The rewards determine the constitutive contribution and ligand-induced contribution by measuring receptor degradation. Firstly, the constitutive contribution for the receptors and ligand-receptor complexes are verified by evaluating the reactions R_p2, R_p3, R_p6 and R_p10. Secondly, the ligand-induced contributions measured by considering the receptors that have been complexed with ligands. Reactions R_p2 and R_p3 signify this process. Figure 6 for CD1(R_p2), CD3 (R_p6) and CD4 (R_p10) demonstrates that receptor degradation has almost similar constitutive contribution for the receptors and ligand-receptor complexes. Figure 6 for CD2 (R_p3) shows receptor degradation has a ligand-induced contribution that affects ligand-receptor complexes. This means that the degradation process of ligand-receptor complexes in plasma membrane is not constantly active as

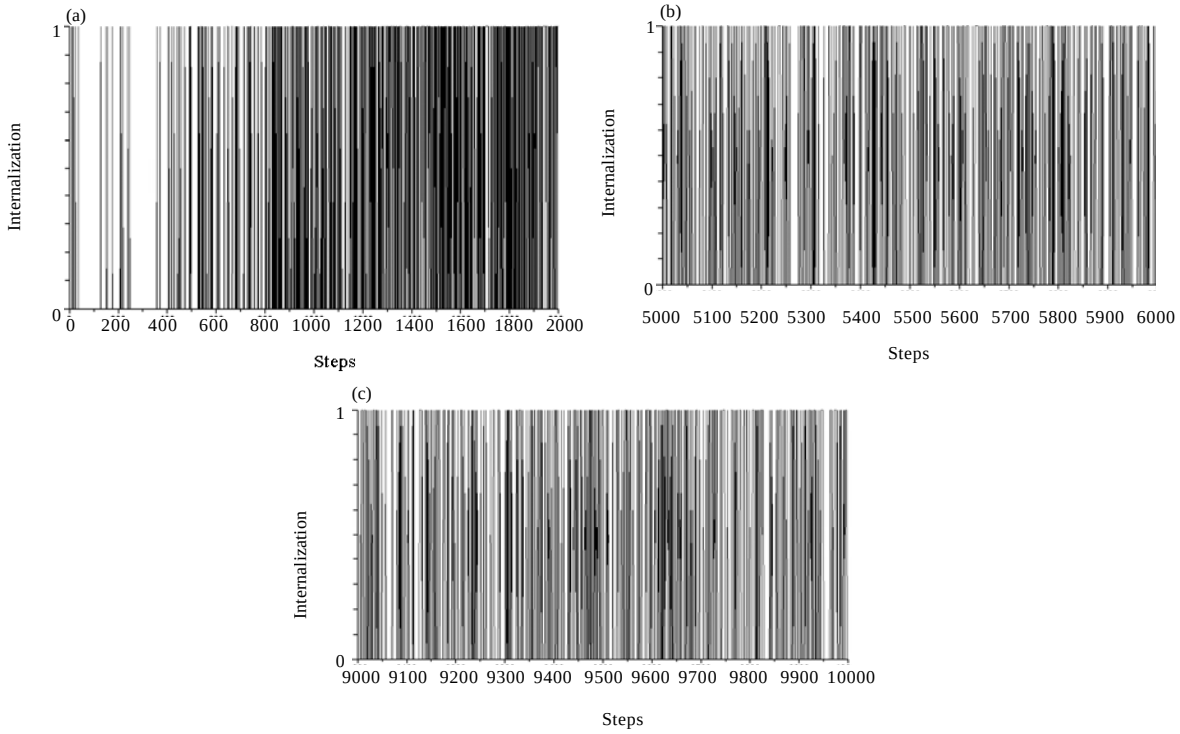

Fig. 4(a-c): Internalization of RI, RII and IRIRII into endosomes for time steps: (a) 0-2000, (b) 5000-6000 and (c) 9000-10000

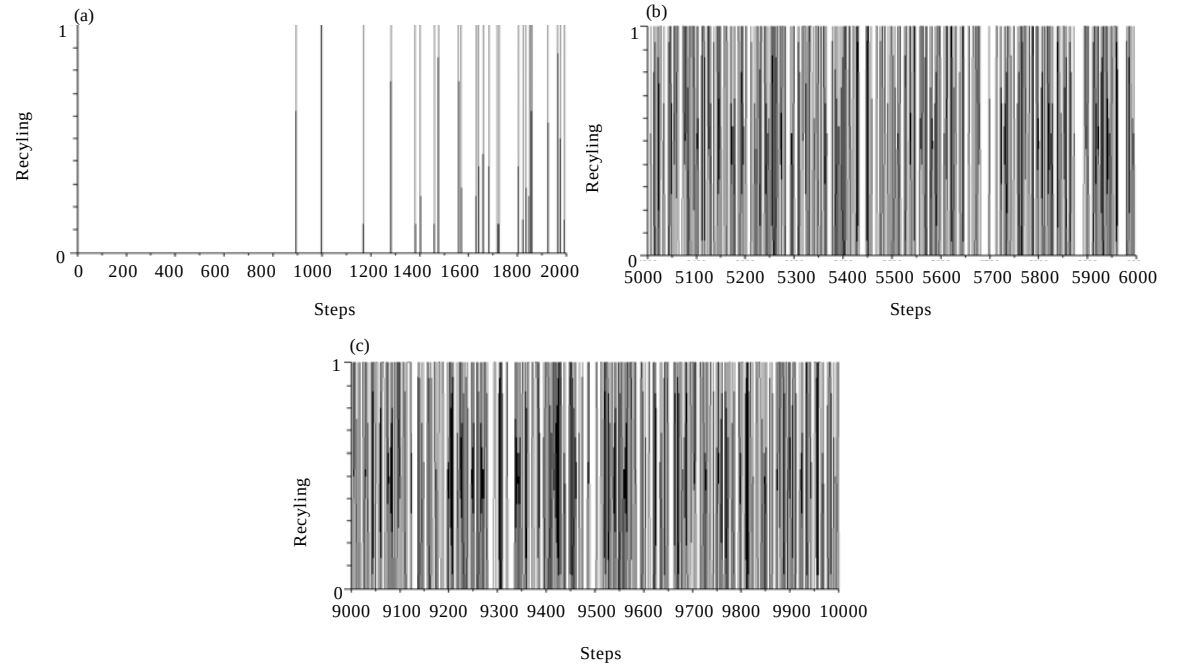

Fig. 5(a-c): Recycling of RI, RII and IRIRII into plasma membrane for time steps: (a) 0-2000, (b) 5000-6000 and (c) 9000-10000

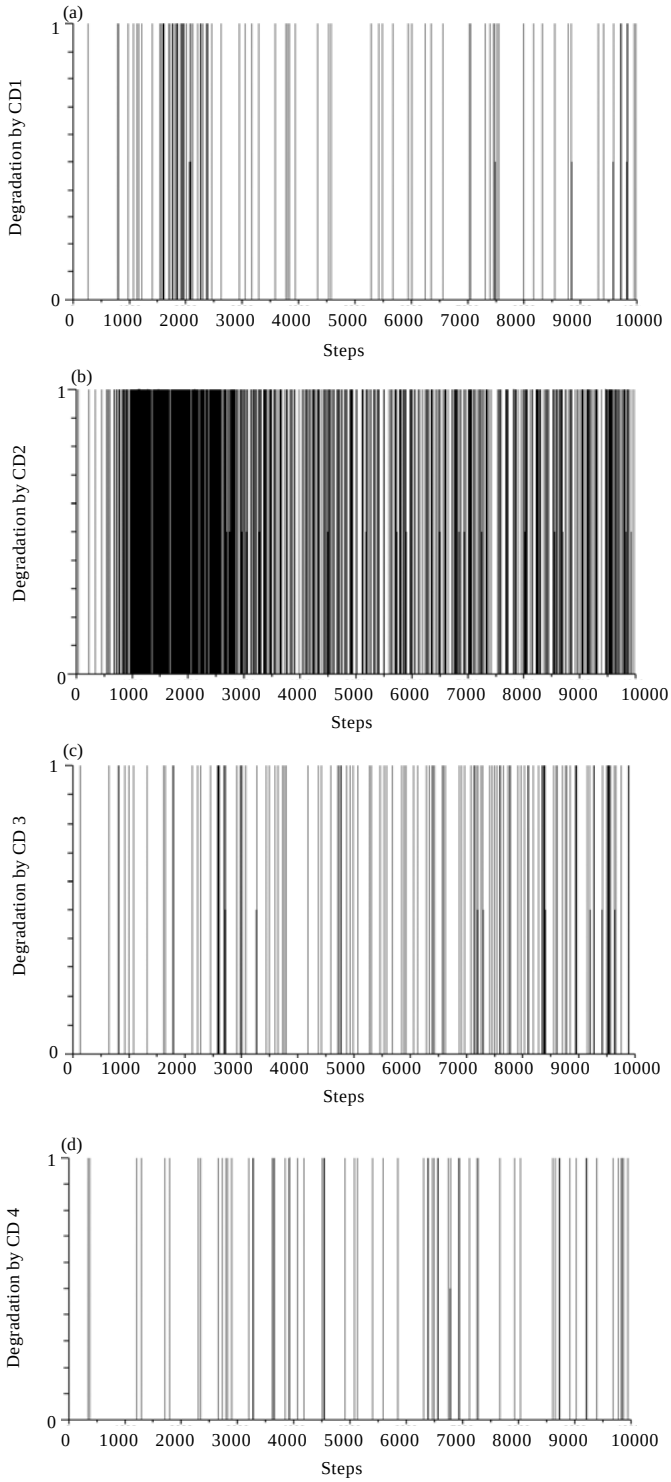

Fig. 6(a-d): Constitutive contribution and ligand-induced contribution caused by receptors degradation for reaction (a) CD_1 (b) CD_2 (c) CD_3 and (d) CD_4

the others but affect more in degradation process of ligand-receptor complexes in plasma membrane.

DISCUSSION

The simulation for membrane computing model of Ligand-Receptor Networks of TGF-β done by Muniyandi and Zin (2011) shows that approximately similar simulation result of ODE model could also be obtained using membrane computing model. The model checking of membrane computing model for Ligand-Receptor Networks of TGF-β demonstrates that the specified properties and behaviours of the system have been conserved in the membrane computing model.

The model checking of property (1) for membrane computing model of LRN system shows the formation of ligand-receptor complex lRIRII in plasma membrane would accompanied by the internalization process to increase the level of lRIRII in endosome until it reached a peak as shown in Fig. 1. But after the peak the level of lRIRII in endosome is suppressed by active recycling process by channelling lRIRII into plasma membrane. The concurrent processes of internalization and recycling ensure the level of availability of receptors and ligand-receptor complexes in plasma membrane and endosome to maintain the behaviour of lRIRII in endosome as shown by the model checking of property (2) in Fig. 2 and 3.

The model checking results of property (3) and (4) further reinforce the complementing activity of internalization and recycling. Figure 4 shows the internalization process rapidly activated around the peak of lRIRII in endosome and Figure 5 demonstrates that the recycling process hardly activated at initial stage but grow rapidly after the peak of lRIRII in endosome. The degradation process of receptors and ligand-receptors complexes model checked with property (5) shows this process essential to consistently suppress the level of receptors and ligand-receptors complexes in plasma membrane to make sure the coordinated processes of internalization and recycling.

The formation, degradation, internalization, recycling and synthesis processes in Ligand-Receptor Networks of TGF-β have been activated according to the properties of the system in the membrane computing model as shown by the model checking of the system. With stochastic selection of the processes for discretely varying the receptors and ligand-receptors complexes levels in the compartments, the signaling activity according to the number of ligand-receptor complexes in the internalized endosomes is measured.

CONCLUSIONS

This study demonstrates that membrane computing with its non-deterministic and discrete characteristics, capable in preserving the properties of Ligand-Receptor Networks of TGF-β. This is possible because membrane computing is abstracting the structure and processes of the system to be represented in a formal way without disregarding its properties. This allows the Ligand-Receptor Networks of TGF-β to be modeled in better way to counter some of the limitations in the conventional method based on ODE.

The stochastic model checking approach of PRISM assists in validating the specification of Ligand-Receptor Networks of TGF-β modeled in membrane computing. This validating method act as complementing approach to the verification method of simulation of the same model as investigated in Muniyandi and Zin (2011). This reinforces that membrane computing provides formal platform in representing and evaluating the network and interaction in biological system.

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REFERENCES

Alur, R. and T.A. Henzinger, 1999. Reactive modules. Formal Methods Syst. Des., 15: 7-48.

De Jong, 2002. Modeling and simulation of genetic regulatory systems: A literature review. J. Comput. Biol., 9: 67-103.

Grumberg, O. and H. Veith, 2008. 25 Years of Model Checking: History, Achievements, Perspectives. Springer, London, UK., ISBN-13: 9783540698494, pp: 36-40.

Kuntz, M. and M. Siegle, 2006. Symbolic model checking of stochastic systems: Theory and implementation. Proceedings of the 13th International SPIN Workshop on Model Checking Software, March 30-April 1, 2006, Vienna, Austria.

Kwiatkowska, M., G. Norman and D. Parker, 2002. PRISM: Probabilistic symbolic model checker. Lecture Notes Comput. Sci., 2324: 113-140.

Kwiatkowska, M., G. Norman and D. Parker, 2007. Stochastic model checking. Proceedings of the 7th International School on Formal Methods for the Design of Computer, Communication and Software Systems, May 28-June 2, 2007, Bertinoro, Italy, pp: 220-270.

Massagu, J., 1998. TGF-beta signal transduction. Annu. Rev. Biochem., 67: 753-791.

Muniyandi, R.C. and A.M. Zin, 2009. Modeling of biological processes by using membrane computing formalism. Am. J. Applied Sci., 6: 1960-1968.

Muniyandi, R.C., A.M. Zin, Z. Shukor, R.C. Muniyandi, A.M. Zin and Z. Shukor, 2010. Model checking the biological model of membrane computing with probabilistic symbolic model checker by using two biological systems. J. Comput. Sci., 6: 669-678.

Muniyandi, R. and A.M. Zin, 2011. Simulation strategy of membrane computing to characterize the structure and non-deterministic behavior of biological systems: A case study with ligand-receptor network of protein TGF-β. Proceedings of the 2nd International Visual Informatics Conference, November 9-11, 2011, Springer, Selangor, Malaysia, pp: 56-66.

Paun, G., 2000. Computing with membranes. J. Comput. Syst. Sci., 61: 108-143.

Raczynski, S., 2006. Modeling and Simulation: The Computer Science of Illusion. Wiley, USA., ISBN-13: 9780470030172, pp: 4-11.

Romero-Campero, F.S, M. Gheorghe, L. Bianco, D. Pescini, M.J. Perez-Jimenez and R. Ceterchi, 2006. Towards probabilistic model checking on P systems using PRISM. Lecture Notes Comput. Sci., 4361: 477-495.

Shi, Y. and J. Massague, 2003. Mechanism of TGF-β signaling from cell membrane to the nucleus. Cell, 113: 685-700.

Steiner, M.S. and E.R. Barrack, 1992. Transforming growth factor-β 1 overproduction in prostate cancer: Effects on growth *in vivo* and *in vitro*. Mol. Endocrinol., 6: 15-25.

Vilar, J.M.G., R. Jansen and C. Sander, 2006. Signal processing in the TGF-β superfamily legend-receptor network. PLoS Comput. Biol., 2: e3-e3.